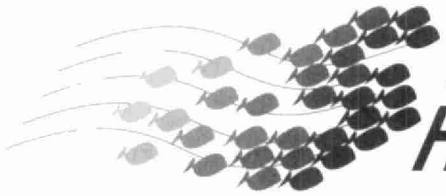

REMEDIAL ACTION PLAN

PLAN D'ASSAINISSEMENT

Fish Tainting Evaluation

Remedial Action Plan
Plan d'Assainissement

Canada Ontario

Canada-Ontario Agreement Respecting Great Lakes Water Quality
L'Accord Canada-Ontario relatif à la qualité de l'eau dans les Grand Lacs

Spanish River Remedial Action Plan

Fish Tainting Evaluation

by

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EXECUTIVE SUMMARY

Fish tainting has historically been a major use impairment in the Spanish River, downstream of the pulp and paper mill at Espanola. Despite recent sensory evaluation studies indicating that this problem has been resolved following improvements to the mill effluent, concerns about the taste and odour of the fish have still been expressed in recent public meetings and by the Spanish River Remedial Action Plan Public Advisory Committee.

An *in situ* fish exposure and subsequent sensory evaluation were conducted in September, 1989 to address these concerns. Caged rainbow trout were exposed directly below the effluent diffuser for 72 hours. Fish were also caged for the same period upstream of the mill to act as controls. A sensory evaluation was conducted using the triangle test to determine if there was a significant difference in odour between the exposed and control fish. The triangle test method involves the presentation of three samples, in which two are the same and one is different. The panelist is required to determine which sample is different. The panel consisted of eight members of the Public Advisory Committee and three members of the Remedial Action Plan team.

The number of correct responses was not significantly different at the 95% confidence level from the chance of correctly "guessing" the odd sample. The panelists were also requested to identify if the odd sample was an exposed or control fish. The number of correctly identified samples was not significantly different at the 95% confidence level from the chance of correctly "guessing" if the sample was an exposed or control fish. The panelists' comments indicate that there was generally no perceived difference between the control and exposed fish. Where differences were noted, the odour was not thought to be objectionable.

In conclusion, the sensory evaluation panel could not distinguish between the exposed and control fish. In addition, the sensory evaluation panel could not distinguish a noticeable taint in those tests where they may have correctly differentiated between the control and exposed samples. There appears to be no tainting potential associated with the effluent from the E.B. Eddy Limited diffuser discharge during the period of investigation.

1.0 INTRODUCTION

1.1 BACKGROUND

The Spanish River downstream of Espanola and the Spanish delta were designated as an Area of Concern by the International Joint Commission (IJC) in 1980. This area is one of the seventeen Canadian Areas of Concern in the Great Lakes for which a Remedial Action Plan (RAP) is being developed.

Fish tainting has been historically identified as one of the most prevalent use impairments throughout this area. Since as early as 1947 complaints have been made by both sport and commercial fisherman about the odour of the water and the foul taste of fish from the Spanish River downstream of Espanola. The taste and odour imparting chemicals were thought to originate from the K.V.P (Kalamazoo Vegetable Parchment) pulp and paper mill effluent (Dymond and De Laporte 1952).

Studies conducted by Swabey in 1965 concluded that the taste and odour of walleye from the lower Spanish River and the North Channel were significantly different from control walleye from Onaping Lake. Sensory panel evaluations carried out in 1980 (OMOE 1983) again confirmed a statistically significant degree of tainting, and concluded that the problem extended as far west as Brennan Harbour. However, commercial catches of fish being taken in the Whalesback Channel at that time were being accepted for market (Running 1983).

In 1983, the pulp and paper mill, now owned by E.B. Eddy Forest Products Limited, embarked on a major modernization and improvement program which included the installation of secondary waste treatment and a steam stripping condensate tower. These changes resulted in a significant improvement in effluent quality.

During the fall of 1985, fifteen anglers fishing the river were randomly surveyed. Twelve indicated they ate the fish they caught and found them comparable with fish from other inland lakes. Two said they found some improvement in quality, and one said he wouldn't eat fish from the river but gave them away to friends.

Another fisheries survey was conducted from August 24 to September 30, 1987 (Bowman 1987). A total of 107 interviews were conducted in three fishing reaches: downstream of Espanola; Spanish Harbour; and Massey. Eighty-eight percent of the respondents stated that they ate the fish

they captured. Of these, 86% rated the flavour of the fish the same as those caught in other lakes and rivers. Four percent rated the flavour as better, and 10% as worse. Some anglers commented that they only ate the smaller walleye because the larger fish were less palatable. Others felt that the walleye were fine, but that the catfish had a muddy flavour. It was concluded that fish tainting in the Spanish River, although improved, was still an identified use impairment.

A third panel evaluation was undertaken in January, 1987, using walleye caught at Cameron Rapids, approximately 20 km downstream of Espanola. Statistical analysis of the sensory evaluation results determined that there was no significant difference in taste and odour between these fish and control walleye collected from Lake Temiskaming (Linquist 1987).

In an April, 1988 telephone survey of area residents (Northern Research Associates 1988), 92% rated the quality of fish as good to excellent. However, 75% of the native peoples and 25% of the Massey residents who had eaten fish from the Spanish River rated the quality of the fish as poor, as compared to 4% from Espanola and 0% from Spanish.

Despite the evidence that the tainting problem has been remediated, occasional concerns about the taste and odour of the fish caught below Espanola have been expressed in public meetings and at the RAP Public Advisory Committee (PAC) discussions held in 1988 and 1989. To address these concerns, and to determine if this use impairment has indeed been resolved, an additional controlled fish tainting evaluation was conducted by the Remedial Action Plan team in September, 1989. This evaluation involved an *in situ* fish exposure and sensory panel evaluation involving members of the PAC and RAP team.

1.2 STUDY OBJECTIVES

The main objective of the study was to determine if there was a significantly different odour in the flesh of caged rainbow trout exposed upstream of the mill and caged trout placed directly adjacent to the mill's diffuser outfall.

A secondary objective was to determine which compounds may be contributing to tainting by analyzing selected fish tissues. This was to be pursued only if there was a statistically significant difference in odour between the two exposed groups of fish.

2.0 METHODS

2.1 FISH EXPOSURE

Rainbow trout were chosen as a test species because they are readily obtained, and have a high fat content. The organic compounds that cause taint tend to be selectively retained in the fat tissues.

Disease free rainbow trout, 20 to 25 cm in length, were purchased from Aquafarms Ltd. in Feversham, Ontario and shipped via air freight to Sudbury. These fish were ultimately transported to an acclimatization facility established upstream of the E. B. Eddy effluent discharge in Espanola. The fish were held in a flow-through enclosure under natural photoperiod for 72 hours subsequent to their transfer. During the period of acclimatization they were fed pelletized feed and were feeding "normally" prior to the field exposure.

The fish exposures were conducted during the week of September 18, 1989. Fish were exposed in floating net pens both in Eddy's upstream mill pond and immediately downstream of the mills discharge. This period was chosen to produce a "worst case" situation because lower flows result in a minimum dilution of the effluent, while the temperature is near the optimum for rainbow trout. Dissolved oxygen, temperature, sodium and conductivity were monitored daily to assess suitability for survival and effluent dilution ratios. The fish were not fed during the exposure period.

After a 72 hour exposure period, the fish were sacrificed and temporarily stored on ice until they were processed on site (within three hours). The lengths and weights of each fish are summarized in Table 1. Bile and liver samples were collected from each fish and individual fillets (including skin) removed for subsequent transport on ice to Sudbury. The fillets were frozen until used in the panel evaluation on September 27, 1989.

The bile and liver samples were frozen and retained for chemical analysis to identify contributing compounds if a significant degree of tainting was confirmed by the panel assessment (Oikari and Holmbolm 1986).

Table 1. Weights and lengths of exposed and control fish.

Fish Type	Weight (gms)	Length (cm)
Exposed		
1	138.0	24.0
2	100.0	21.5
3	110.0	22.0
4	131.0	23.5
5	106.0	21.0
6	127.0	22.5
7	73.0	19.0
8	67.0	19.0
Control		
1	118.0	22.5
2	107.0	21.0
3	107.0	22.0
4	122.0	22.0
5	113.0	22.0
6	102.0	21.0
7	92.0	20.5
8	67.0	18.5
9	70.0	20.0
10	130.0	22.5

2.2 TAINTING EVALUATION

2.2.1 Sample Preparation

The flesh of all fish from each exposure were minced together in a glass Osterizer blender. The skin was kept on the fillets to more closely emulate actual consumption conditions, as rainbow trout is frequently cooked without removing the skin. In addition, many of the organic compounds associated with tainting are associated with the lipophilic fat layer just below the skin. Combining all fillets eliminated any odour variability between individual fish. The fish fillets were kept partially frozen to allow better mixing, and to minimize the loss of any volatile compounds.

Five gram aliquots (±0.5 gm) were weighed on a Sauter top-loading balance, and immediately wrapped in aluminum foil packets of 80 mm by 40 mm folded dimensions. Approximately equal proportions of muscle and skin were included in each sample. A three-digit code was marked on each packet with a wax pencil for identification during sensory analysis. The packets were then vacuum sealed in polyethylene bags using a Decosonic™ (No. 828) Vacuum Bag Sealer. Three packets were sealed in each bag, corresponding to each triangle test set. The set number was marked in wax pencil on the outside of the bag.

Care was taken that the tissue was exposed to the air as little as possible during the blending and weighing of the individual samples to minimize the loss of any volatile compounds. Only glass and stainless steel instruments were used in the preparation of the samples. Soap was not used by the investigators preparing the samples either prior to or during the preparation procedure.

2.2.2 Sensory Evaluation

The assessment of taint was conducted using a sensory evaluation method called the triangle test. This method is currently being advocated by international agencies as the most useful and reliable test for identifying possible tainting in fish (Tidmarsh and Ackman 1986). In the triangle test, three samples are presented to the panelist. Two of the samples are the same, and one is different. The panelist judges which sample he or she believes is different. This is a forced choice method; the panelist cannot abstain from a decision even if he or she does not detect any difference between the samples. The triangle test has the advantage of having a smaller statistical probability of a panelist "guessing" whether a sample is tainted or untainted (i.e. 33%, as opposed to 50% for a test involving the comparison of two samples). Forced choice, three sample tests are the most

sensitive sensory testing methods, and hence are most applicable where the expected difference between samples is slight (A.S.T.M. 1968).

A modified version of the triangle test was used in this study, in which the panelist was further required to state whether the odd sample was tainted or untainted, and provide any comments and/or descriptors for each sample set. This modification was first used by Cohen et al. (1960).

Ideally, six sets of three samples should be used to produce a balanced design of every possible presentation order combination of control and exposed fish. Unfortunately, there was a limited amount of fish tissue, and only five sets of three samples were presented to each panelist. Each set consisted of either one sample of exposed fish and two of control fish, or one sample of control fish and two of exposed fish. Each combination of exposed and control samples was different. The order of presentation of each combination was assigned randomly.

The panel sessions were held in the water analysis laboratory of the Northeastern Regional Office in Sudbury. Four evaluations were made at one time. The panelists consisted of eight members of the PAC and three members of the RAP team. All panelists were non-smokers. The PAC had been informed of the background, objectives and procedures for the tainting study, and were requested to participate in the sensory evaluation, through an information sheet provided prior to the session. The criteria for participation were: a) panelists must like fish; b) panelists should be non-smokers; c) panelists should refrain from eating or drinking (other than water) for 30 minutes prior to each session; and d) panelists should avoid the use of perfume, aftershave or scented soaps on the day of the sensory evaluation.

The sealed samples were placed in 1 L beakers containing approximately 750 mL of hot water. These beakers were placed on hot plates and heated to a temperature of 60°C. The samples remained sealed in the polyethylene bag from the time of preparation until opened by the panelist for evaluation. This ensured that there was no loss of volatile compounds prior to the sensory evaluation. Panelists were requested to remove only one packet from the hot water at a time, and to complete their evaluation of this set of samples before proceeding to the next set. This ensured that optimum heat was retained in the samples. Completed samples were removed after each evaluation to prevent cross-over of odours between samples.

Panelists were given a dilute lemon solution (approximately 1 tbsp. lemon juice to 1 L of room temperature water) as a rinsing agent, and unsalted soda crackers were provided to clear the palate between samples. Panelists were requested to evaluate which sample was different from the other

two. A decision was required even if they could not detect a difference. Panelists were further requested to indicate whether the odd sample was "tainted" or "untainted", and to provide any comments on the evaluation or odour of the samples. If no difference was detected, this was to be indicated in the comments section. Because of the unknown nature of any compounds in the fish tissue, an odour evaluation only was required. The questionnaire and instructions provided to the panelists are given in Figure 1.

3.0 RESULTS AND DISCUSSION

3.1 PHYSICAL AND CHEMICAL WATER QUALITY

Physical and chemical water quality data collected during the fish exposure period are summarized in Table 2. Water temperatures were between 18°C and 19°C at all instream locations. Effluent temperatures were between 31°C and 32°C. Dissolved oxygen concentrations were between 8.2 and 9.1 mg/L in the river, and between 6.8 and 7.0 mg/L in the foam pond. Temperatures and dissolved oxygen concentrations were thus suitable for rainbow trout during the exposure period.Sodium is commonly used as a tracer of pulp mill effluents because it is a component of the chemical compounds used in the kraft pulping process. Effluent dilution ratios were estimated based on calculated sodium mass balances. Dilution ratios ranged from 32:1 to 40:1 immediately above the diffuser at the exposure site. Dilution ratios two km downstream of the diffuser ranged from 46:1 to 51:1, assuming total mixing.

3.2 SENSORY RESULTS

For the purpose of this discussion, a "correct response" or "correct judgement" means the panelist correctly determined which sample of the three presented was different from the other two. A "correct identification" means the panelist correctly identified the different sample as a control or exposed fish. Correct identifications are only applicable to those samples that were correctly judged as different (Larmond 1977).

Of the 55 triangle tests conducted (five sets evaluated by each of eleven panelists), the "different" sample was correctly identified on 21 occasions. Analysis of the results of a triangle test is based on the probability that if there is no detectable difference, the odd sample will be selected by chance one-third of the time (A.S.T.M. 1968; Larmond 1977). Therefore, the results for each test were compared with a chance probability of 33% using a t-test for paired data. The number of correct responses was not significantly different at the 95% confidence level from the chance of

SPANISH RIVER REMEDIAL ACTION PLAN
FISH TAINTING ODOUR EVALUATION

NAME _____ DATE _____

PRODUCT _____

Five sets of three coded samples are provided. Each set should be evaluated separately, in the order listed. In each set, two of the three samples are identical, and the third is different.

To evaluate the samples, tear off the end of the foil packet and open it as much as possible. Sniff the contents three times, then fold the end of the packet to reclose the packet. Wait approximately 15 seconds, and repeat the procedure for the other two samples in the set. Test the samples separately and in the order indicated. Check the odd sample and indicate whether the odd sample is tainted or untainted. Record any additional comments you may have describing the odour of the sample(s) you believe is (are) tainted.

Rinse between each sample with the water provided and take a bite of cracker after smelling each sample.

	Code	**Check odd sample**	**Odd sample is: (check one)**	**Comments**
SET #1	____	_____	Tainted ____	_____
	____	_____	Untainted ____	_____
	____	_____		_____
SET #2	____	_____	Tainted ____	_____
	____	_____	Untainted ____	_____
	____	_____		_____
SET #3	____	_____	Tainted ____	_____
	____	_____	Untainted ____	_____
	____	_____		_____
SET #4	____	_____	Tainted ____	_____
	____	_____	Untainted ____	_____
	____	_____		_____
SET #5	____	_____	Tainted ____	_____
	____	_____	Untainted ____	_____
	____	_____		_____

Figure 1. Sensory evaluation questionnaire

Table 2. Physical and chemical water quality during the fish exposure period.

	Upstream Cage	Foam Pond	Downstream Cage	Two km Downstream
September 19				
Temperature (°C)	19.0	32.3	18.0	18.9
Dissolved Oxygen (mg/L)	8.9	7.0	8.5	9.1
Sodium (mg/L)	2.7	260.0	11.0	7.1
Conductivity (umhos/cm)	71	1620	124	100
Flow (m^3/sec)	56.41	1.11	57.52	-
Dilution Ratios for Sodium*	-	-	32:1	51:1
September 20				
Temperature (°C)	18.0	31.0	18.5	18.3
Dissolved Oxygen (mg/L)	8.7	6.8	8.3	8.4
Sodium (mg/L)	2.7	280.0	11.0	7.4
Conductivity (umhos/cm)	72	1700	123	103
Flow (m^3/sec)	54.77	1.16	55.93	-
Dilution Ratios for Sodium*	-	-	32:1	47:1
September 21				
Temperature (°C)	18.0	-	18.0	18.5
Dissolved Oxygen (mg/L)	8.7	-	8.2	8.3
Sodium (mg/L)	2.7	290.0	10.0	8.8
Conductivity (umhos/cm)	74	1750	125	114
Flow (m^3/sec)	61.14	1.33	62.47	-
Dilution Ratios for Sodium*	-	-	40:1	46:1
September 22				
Temperature (°C)	18.1	-	18.5	18.5
Dissolved Oxygen (mg/L)	8.8	-	8.3	8.2
Sodium (mg/L)	2.7	290.0	11.0	9.2
Conductivity (umhos/cm)	72	1740	124	113
Flow (m^3/sec)	58.73	1.25	59.98	-
Dilution Ratios for Sodium*	-	-	39:1	47:1

* Estimated using calculated mass balances. Assumes total mixing at the station two km downstream.

correctly "guessing" the odd sample (p=0.4635). It is thus concluded that the sensory evaluation panel could not distinguish between the exposed and control fish.

Of these 21 correct responses, 12 samples were correctly identified as either the control or exposed fish. The correct identifications for each correct response were compared with a chance probability of 50% using a paired t-test. The number of correctly identified samples was not significantly different at the 95% confidence level from the chance of correctly "guessing" if the sample was a control or exposed fish (p=0.5259). The conclusion is that the sensory evaluation panel could not distinguish a noticeable taint in those tests where they may have correctly differentiated between the control and exposed samples.

Figure 2 illustrates the number of correct responses for each panelist. In addition, the number of these samples correctly identified as the exposed or control fish is shown. Panelist #6 was the most sensitive member of the panel, correctly identifying the odd sample in four out of five tests, and correctly identifying the nature of the exposure in three out of these four responses. Panelist #7 also responded correctly in four out of the five test sets, and correctly identified two of the four samples.

Figure 3 illustrates the number of correct responses for each triangle test set. The letters indicated for each test correspond to the order of presentation of the samples. For example, in set #1 the samples presented were an **E**xposed fish, then a **C**ontrol fish and finally an **E**xposed fish. Also shown are the number of correct responses that were correctly identified as the exposed or control fish. Set #3 (CCE) was most frequently judged correctly, with six out of eleven correct responses, and five out of six correct exposure identifications. Set #2 (CEC) also had six out of eleven correct responses, with four out of the six correctly identified. In general, the sample sets in which a control fish was presented first were most frequently judged correctly. A secondary factor in correct identification appears to be the use of two controls and one exposed sample in a test set. These observations are, however, based on a small sample size, and would require a larger number of evaluations for confirmation.

The comments made by the panelists are summarized in Table 3. In general, the comments indicate that the panelists could not differentiate between the exposed and control fish, and that many of their responses were "guesses". Where a different odour was detected, it was not perceived to be objectionable.

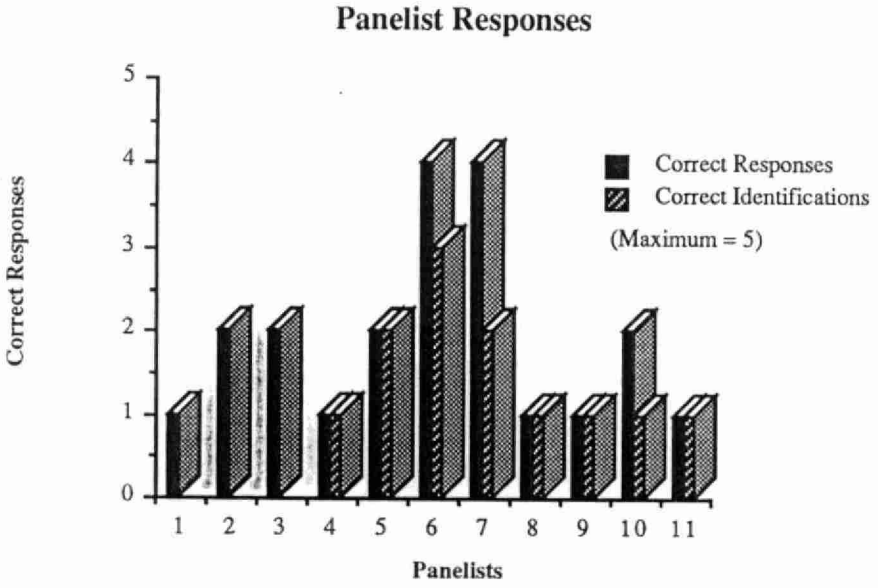

Figure 2. Correct responses and correct identifications for each panelist.

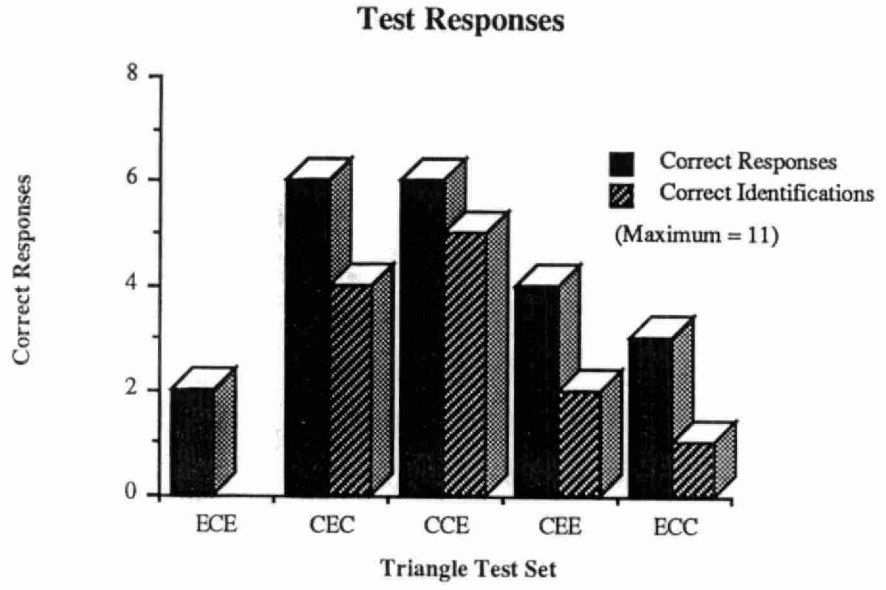

Figure 3. Correct responses and correct identifications for each triangle test set.

Table 3. Summary of sensory evaluation results.

Panelist	Test Set No.	Correct Response	Correct Identification	Comments
1	1	Yes	No	different odour
	2	No	-	slightly different
	3	No	-	odour
	4	No	-	odour
	5	No	-	chemical odour
2	1	No	-	(none)
	2	No	-	(none)
	3	Yes	No	(none)
	4	No	-	(none)
	5	Yes	No	(none)
3	1	No	-	(none)
	2	Yes	No	(none)
	3	No	-	(none)
	4	Yes	No	(none)
	5	No	-	(none)
4	1	No	-	different, not bad, stronger "fish" smell
	2	No	-	couldn't tell them apart
	3	Yes	Yes	stronger "fish" smell, but not bad
	4	No	-	as in #3
	5	No	-	as in #3
5	1	No	-	no substantial difference could be
2	Yes	Yes	noticed throughout	
	3	Yes	Yes	"
	4	No	-	"
	5	No	-	"
6	1	No	-	couldn't really discern a difference
	2	Yes	Yes	"
	3	Yes	Yes	"
	4	Yes	Yes	"
	5	Yes	No	"
7	1	Yes	No	(none)
	2	Yes	No	(none)
	3	Yes	Yes	(none)
	4	No	-	(none)
	5	Yes	Yes	(none)

continued . . .

Table 3. Concluded.

Panelist	Test Set No.	Correct Response	Correct Identification	Comments
8	1	No	-	(none)
	2	No	-	(none)
	3	No	-	(none)
	4	Yes	Yes	(none)
	5	No	-	(none)
9	1	No	-	(none)
	2	No	-	(none)
	3	Yes	Yes	(none)
	4	No	-	(none)
	5	No	-	(none)
10	1	No	-	did not find any noticeable difference
	2	Yes	Yes	- all were very palatable
	3	No	-	"
	4	Yes	No	"
	5	No	-	"
11	1	No	-	guessing
	2	Yes	Yes	guessing
	3	No	-	guessing
	4	No	-	faint odour
	5	No	-	faint odour

Correct Response - Odd sample was correctly determined.

Correct Identification - Odd sample was correctly identified as control or exposed fish

"-" - Identification is not applicable because sample was not judged correctly

As no significant taint was determined from the sensory evaluations, the chemical analyses and the bile, liver and muscle tissue was not conducted. Such analyses would only have been useful if the results could be correlated to organoleptic perceptions.

4.0 CONCLUSIONS AND RECOMMENDATIONS

The sensory evaluation panel could not distinguish between the exposed and control fish. In addition, the sensory evaluation panel could not distinguish a noticeable taint in those tests where they may have correctly differentiated between the control and exposed samples. The panelist comments indicate that there was generally no perceived difference between the control and exposed fish. Where differences were noted, the odour was not thought to be objectionable. In conclusion, there appears to be no tainting potential associated with the effluent from the E.B. Eddy Limited diffuser discharge during the period of investigation.

It can be reasonably concluded that the process and effluent treatment changes instituted by the mill have essentially eliminated the compounds that previously caused off-flavours in the fish of the Spanish River. The continued perceived tainting problem associated with the pulp mill effluent appears to be an artifact of the long history of fish tainting in the river. The muddy-earthy flavour noted in catfish in the 1987 fisheries survey may be associated with the presence of naturally occurring actinomycetes and blue-green algae in the water (Iredale and York 1976). However, this off-flavour was not noted in this study.

It is recommended that efforts be made to provide information to the public about the significance of the mill process and treatment changes, and the results of this and other recent tainting studies. This knowledge will help to dispel any persistent misconceptions about continued tainting from the mill effluent.

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